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ON THE CHROMOSOME NUMBERS OF HUMAN AMNION CELLS IN PRIMARY AND STRAIN CULTURES*

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Cell cultures from human amnion recently have attracted considerable attention. The report of the establishment of a cell which persisted as an easily manageable "strain" by Fogh and Lund (1957) was followed by descriptions of morphological events occurring in the course of the formation of other lines by Dunnebacke (1956). Fernandes (1958) launched a similar line with the important features that within 35 days it appeared to have acquired the rapidly proliferative and nutritionally facultative characteristics which are generally ascribed to this class of cells.

Amnion cell strains have been reported to be particularly useful for virological studies. Pomerat et al. (1958) suggested that giant cell formation in amnion and other cell lines as a result of irradiation might be related to the elevated number of chromosomes typical for some of the cells in this organ.

The present study was undertaken with the hope of providing a more detailed chromosomal analysis of primary and strain cultures of a human amnion than those reported in the previous papers (Fernandes, 1958; Pomerat et al., 1958) with a description of the morphology of their chromosomes.

Materials and Methods

Placental tissue from a healthy woman was obtained from the John Sealy Hospital in Galveston, Texas.

Test objects consisted of 3 types of amnion derivatives:

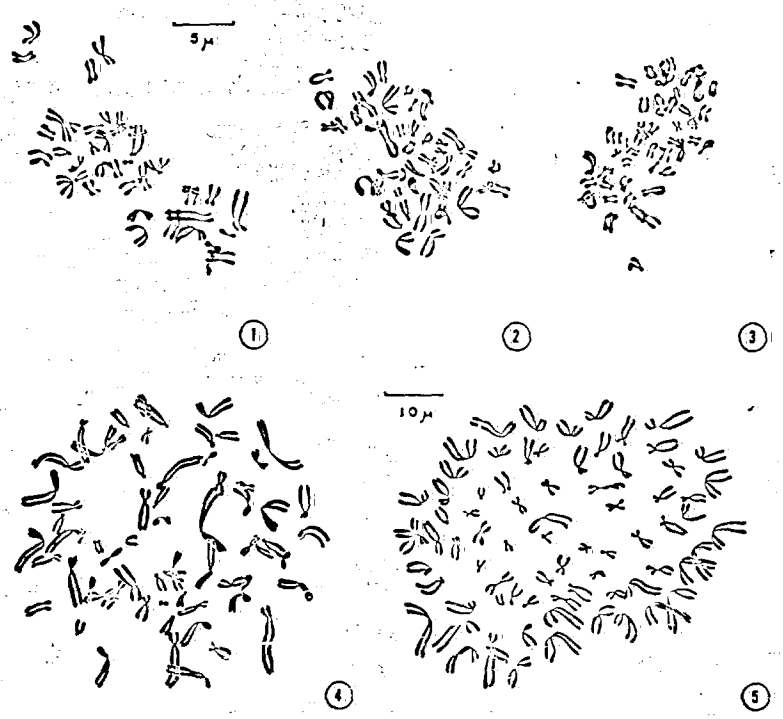
I. *Freshly isolated elements before culture* (cf., figs. 1–3). Using amnion obtained immediately upon delivery, about 150 fragments obtained from various areas measuring approximately 1 mm. on a side were employed for making crush preparations stained with acetic orcein after treatment with a hypotonic solution (1 part of Gey's balanced salt solution plus 9 parts of ordinary tap water) for one hour.

II. *Epithelial cells in primary cultures of amnion tissue.* After 25 days of incubation, cells on cover glasses in roller tubes which had not been freed

with trypsin solution were treated with a hypotonic solution for 10 minutes, fixed with methyl alcohol and stained according to Jacobson's method (Hsu and Pomerat, 1953; Nakanishi, in press).

III. *The development of the Fernandes amnion strain of epithelial cells* (cf., figs. 4–5). This resulted from the trypsinization of amnion tissue and its cultivation without embryonic extract (Fernandes, 1958; Pomerat et al., 1958). On the 35th day from the date of the original trypsinization the chromosome numbers were counted in Rose chamber cultures of the 2nd passage using the same method as that applied for primary cultures. Later a newer technique as described by Hsu and Klatt (1958), Nakanishi and Mizutani (1959) and Nakanishi (in press) was utilized to observed the chromosomes of this strain.

Eagle's medium was employed with the addition of 10 per cent horse serum for primary cultures and for the establishment and maintenance of the strain. At the present time this strain is in the 97th passage, which is over two years after the date of the original trypsinization (April 1957 to May 1959).



FIGS. 1 to 5. Camera lucida drawings of the chromosomes of human amnion cells. FIGS. 1 to 3—freshly isolated cells before culture (42, 48 and 55 chromosomes, respectively). FIGS. 4 to 5—chromosomes of the strain cultures (67 chromosomes in the 2nd subculture and 75 elements in the 84th subculture). The magnification for FIGS. 1 to 4 is given by the scale shown in figure 1 and for figure 5 as shown.

Results and Discussion

I. *The chromosomes of freshly isolated amnion cells.* Walker (1958) found diploid, tetraploid and octoploid numbers of chromosomes in the transitional epithelium of the bladder of mice. For the bladder of newborn mice in which there was a wide range of nuclear size, he reported that out of 139 cells 68 were diploid, 63 were tetraploid and 8 were octoploid. It was concluded that polyploidy and large nuclei in newborn mice were related to normal differentiation by means of which "dome" cells in the surface layer of the transitional epithelium were produced. On the basis of both chromosome counts and modifications of nuclear size, Sachs and Shelesnyak (1955) studied the occurrence of polyploidy in the development of the deciduomata and its absence when this organ was suppressed byan antihistaminic agent. These authors concluded that polyploidy might be related to some specialized activity in the maternal placenta and that since these cells could not reproduce themselves normally or persist, this might provide a possible basis for the limited life of the deciduoma.

In a cytological study of the regenerating rat liver, Makino and Tanaka (1953) and Tanaka (1953) found that while there was a wide variation in the chromosome number of regenerating liver cells from adult rats during restoration after partial extirpation, the most frequent numbers fell in three ranges: diploid (36 to 46 chromosomes), triploid (59 to 68 chromosomes) and tetraploid (78 to 89 chromosomes). However, those in the diploid range were the most numerous.

In order to check the possibility that some areas of the human amnion normally contain cells with more than the diploid number, it was necessary to establish the chromosome count for freshly isolated elements before culture. Unfortunately, the amnion cells obtained directly from fetal membranes showed very little divisional activity. Only 3 cells revealed chromosomes sufficiently well spread to permit accurate counting. The values obtained were 42, 48 and 55 (Figures 1, 2, 3). Even though the chromosomes of only 3 cells were counted, this observation was suggestive of a diploid range for the human amnion.

II. *The chromosomal status of cells in primary cultures.* The chromosome numbers counted in both primary and strain cultures are presented in figure 6. Results for the 24 cells with well-

spread chromosomes obtained in material fixed on the 25th day of *in vitro* life showed a range from 41 to 52 with the highest values at 46 (25%) and 48 (21%).

Since Painter (1923) reported that the chromosome number in man was 48 and Oguma and Kihara (1923) stated that there were 47 chromosomes in the male, a number of workers have challenged these counts. The difficulties encountered in the enumeration of human chromosomes are obviously due mainly to the techniques associated with the handling of cells containing large numbers of these structures. The development of methods for the spreading of chromosomes in cell culture has made possible more critical studies of their number and morphology. Hsu (1952) reported 48 chromosomes in the material from tissue culture. However, he now states (Hsu, 1959) that even with hypotonic solution treatment which greatly enhances the spreading of the chromosomes, his original observation was incorrect. Recently, Tjio and Levan (1956) and Tjio and Puck (1958) found 46 chromosomes for cells of this species in tissue cultures. Further, Kodani (1958) reported that 46, 47 and 48 chromosomes occurred in testicular material. Nakanishi (in press) has studied the chromosome number of cells from human fetal lung tissue. In primary culture he found the most frequent chromosome numbers to be 46 (25%), 47 (22%) and 48 (22%). These values closely approximated those obtained in the course of the present study. Painter (post-graduate seminar, University of Texas, 1958) has discussed Kodani's suggestion that it seemed certain that humans might show either 46, 47 or 48 chromosomes, the variation in number probably being due to the presence of a definite chromosome pair, a univalent, or the absence of an entire pair and pointed out that chromosome "fusion" was quite common in nature.

The newer techniques which have been applied for the observation of chromosomes in tissue cultures (cf., Hsu and Klatt, 1958; Nakanishi and Mizutani, 1959; Nakanishi, in press and in this paper) may be helpful in settling the vexatious uncertainty of the somatic number for man.

III. *Data on chromosomes in strain cultures.* Counts made from 32 cells of the Fernandes amnion strain fixed on the 35th day of incubation had a range from 60 to 77 with a peak at 67 (subtriploid number). Cells in the 40th subculture (195 days

from the date of the initial trypsinization) showed a range from 58 to 90 with a peak at 66. In the latest observations of 50 metaphase plates in the 84th subculture (664 days) the most frequent chromosome number was 75 (31 out of 50 cells) in a narrower range of 69 to 78. In these subcultures the chromosome counts apparently were in the triploid range and no diploid number was found (Figure 6). Examples of excellent metaphase plates which facilitated the examination of chromosomes are presented for reference (Figures 4, 5, 7, 8).

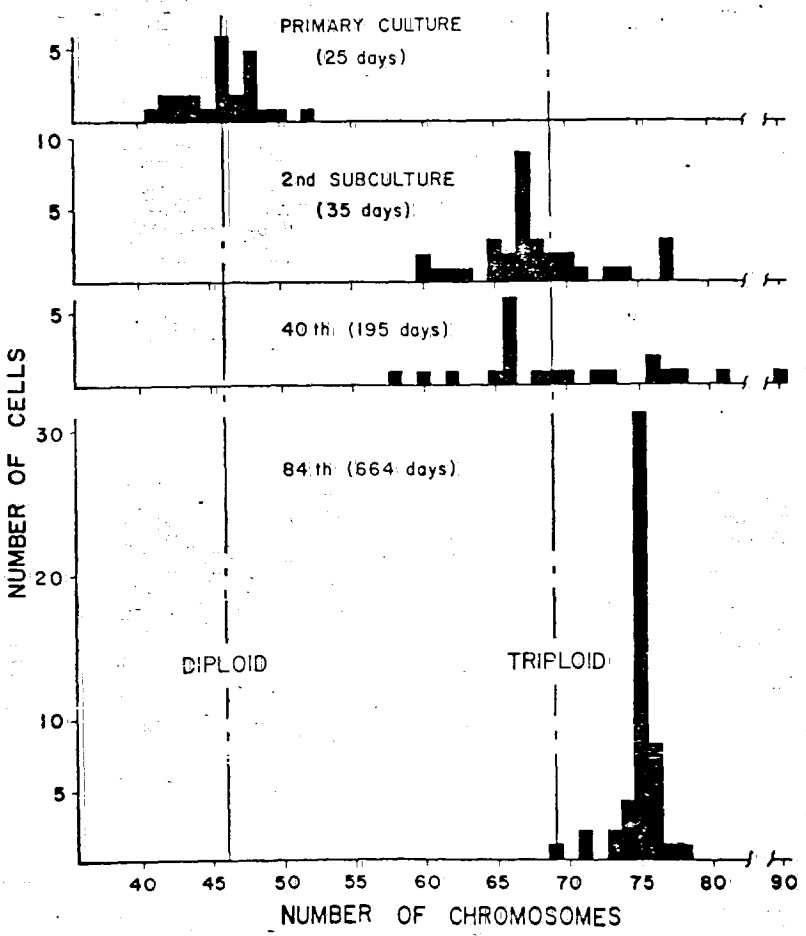
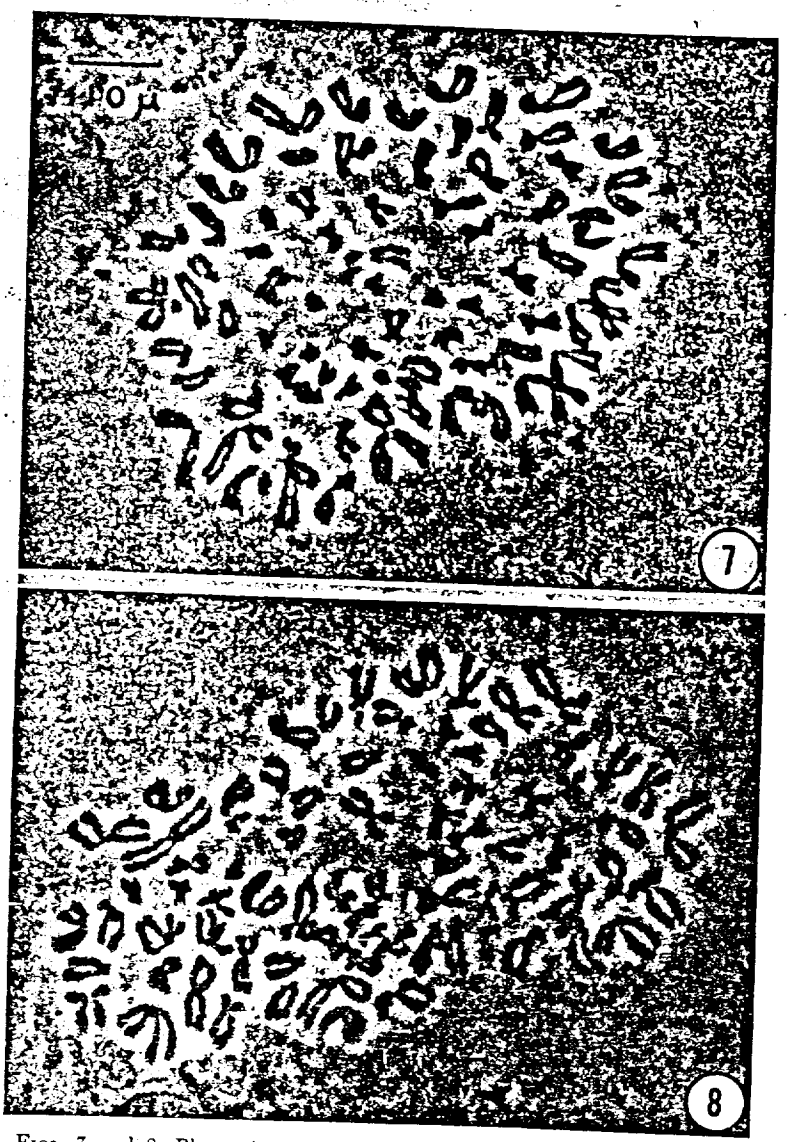


FIG. 6. Distribution of chromosome numbers in human amnion cells in primary and strain cultures.



FIGS. 7 and 8. Photomicrographs of chromosomes of the human amnion cell strain (75 chromosomes, respectively). FIG. 7 corresponds to figure 5. The magnification for figures 7 and 8 is given by the scale shown in figure 7.

As reported by many workers (cf. Levan, 1956; Hsu et al., 1957; Berman et al., 1957; Hsu and Klatt, 1959; Levan and Biesele, 1958; Nakanishi, in press) after continuous cultivation *in vitro*, cells of both normal and malignant origin exhibit extensive heteroploidy with very few or no euploids. Two human lung cell strains have been described (Nakanishi, et al.). The most frequent chromosome numbers were 76 and 77. In contrast, Syverton (1957) reported that in a strain of human tissue only diploids were found after many transfers. Recently, Tjio and Puck (1958) also stated that the chromosome number of cells from normal human skin, cervix and uterus remained constantly at 46 after more than 5 months of continuous, rapid growth in tissue culture involving scores of vessel transfers and a number of generations equivalent to many billions of progeny. Furthermore Ford and Yerganian (1958), in their studies on Chinese hamster cells, found that the diploids coexisted with the heteroploids for a long period.

Since the chromosome count made on the 35th day after the original trypsinization indicated a subtriploid number and also because the multiplicative rate of the cells was very high at this time, it was assumed that the strain was established before the 35th day (Fernandes, 1958). Levan and Biesele (1958) have reported that cells separated by trypsinization showed a higher incidence of chromosomal breaks than those which were mechanically minced. Further, they suggested that the reason trypsinization acted favorably in the establishment of new cell lines might be due to the appearance of cells with changed karyotype from the very beginning of tissue culture. The present observations would serve to supplement these data.

Summary

The chromosome numbers of human amnion cells were studied. The values obtained in freshly isolated elements before culture were 42, 48 and 55. The most frequent chromosome numbers in 25-day primary tissue culture preparations were 46 (25%) and 48 (21%). Counts made from cells of trypsinized cultures fixed on the 35th day of incubation (2nd subculture) had a range from 60 to 77 with a peak at 67. Cells in the 40th subculture (195 days from the date of the initial trypsinization) showed a range from 58 to 90 with a peak at 66. In the latest

observations in the 84th subculture (664 days) of the Fernandes amnion strain, the most frequent chromosome number was 75 (62%) in a narrower range of 69 to 78. The problem of determining the precise somatic chromosome number for man is discussed.

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